RESEARCH ARTICLE

Mechanism of Danshensu on multidrug resistance in MCF-7/DOX cells

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Breast cancer represents a major global health issue with multidrug resistance (MDR) posing a significant challenge. MDR leads to cancer cells becoming resistant to various drugs, severely compromising the effectiveness of breast cancer treatments. Doxorubicin (DOX) is a fundamental drug in the treatment of breast cancer but contributes to the development of MDR. DOX is commonly used in the study of MDR with the overexpression of ATP binding cassette transporter superfamily B member 1 (ABCB1) gene being one of the main mechanisms through which tumor cells exhibit MDR. Current research, including more than 150 clinical trials on ABCB1 inhibitors, aims to overcome this obstacle. However, due to severe side effects and poor solubility, none of these inhibitors have been approved by the U.S. Food and Drug Administration (FDA), which highlights the urgent need for safer and more effective solutions. Danshensu (DSS) shows potential in reversing MDR without the severe side effects associated with other treatments, underscoring its importance in advancing breast cancer therapy. This study investigated the reversing effects and mechanisms of DSS on MDR in human breast cancer cell lines MCF-7/DOX and MCF-7. The 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was employed to evaluate the impact of DSS on cell drug sensitivity and resistance. Further, fluorescence spectrophotometry was applied to measure the intracellular concentration of DOX, and flow cytometry was used to analyze the expression of P-glycoprotein (ABCB1), a protein associated with drug resistance. The results showed that DSS significantly reduced the IC_{50} value of DOX for MCF-7/DOX cells without notable effects on MCF-7 cells, indicating a specific action against resistant cells. Furthermore, DSS increased the intracellular concentration of DOX in MCF-7/DOX cells and decreased the expression of ABCB1 in these cells. The results confirmed that DSS could reverse MDR in MCF-7/DOX cells by reducing the expression of ABCB1. This discovery offered new insights into the mechanisms through which DSS impacted drug resistance and provided a potential therapeutic strategy for overcoming resistance in breast cancer treatments.

Keywords: multidrug resistance; Danshensu; ATP binding cassette transporter superfamily B member 1; IC_{50}; reversal agent.

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Introduction

Breast cancer stands as the predominant cancer among women globally and the leading cause of cancer-related mortality in females worldwide. Present therapeutic strategies for breast cancer encompass surgery, radiotherapy, and chemotherapy. However, a significant challenge arises when patients develop multidrug resistance (MDR) during chemotherapy, often resulting in therapeutic failure. It is thus critical to investigate the molecular mechanisms underlying resistance development in breast cancer and identify resistant targets. This

exploration is vital for offering substantial research and theoretical basis to devise strategies that counteract resistance in breast cancer therapies [1, 2]. MDR refers to the phenomenon where tumor cells become resistant to multiple drugs with varied pharmacological activities and structures, and it is one of the primary factors affecting the efficacy and prognosis of tumor chemotherapy [3]. Doxorubicin (DOX), also known as Adriamycin (ADM), belongs to the anthracycline class of drugs and is a broad-spectrum anti-tumor drug [4]. Long term action of DOX on tumor cells can lead to drug resistance. Research on the multidrug resistance of tumor cells to DOX has become a hot topic [5]. The overexpression of ATP binding cassette transporter super-family B member 1 (ABCB1) gene is one of the main mechanisms by which tumor cells develop MDR [6]. ABCB1 can transport various substrates with different structures and has a "drug pump function" [7]. When tumor cells are exposed to anti-cancer drugs for a long time, the MDR gene is induced to expand and express ABCB1 in large quantities [8]. In this way, tumor cells pump chemotherapy drugs from the inside of the cell to the outside of the cell to form MDR [9]. To some extent, the MDR phenotype is a result of ABCB1 overexpression. MDR reversal agents can compete with anti-tumor drugs for binding sites on ABCB1, reducing the intracellular drug efflux ability of MDR cells, increasing drug accumulation, and restoring cell sensitivity to drugs [9]. To date, three generations of inhibitors targeting ABCB1 have been developed. Despite over 150 clinical trials conducted on more than 30 ABCB1 inhibitors, none have been approved by the U.S. Food and Drug Administration (FDA). The major drawbacks of these ABCB1 inhibitors are their significant toxic side effects, poor solubility, and the alteration of the pharmacokinetic properties of chemotherapeutic drugs, making their widespread application challenging [10, 11]. Verapamil is the first generation discovered ABCB1 inhibitor, but its clinical application is limited due to its serious toxic side effects such as cardiovascular toxicity and nephrotoxicity. However, it is still used as a control in multi drug resistance studies. Therefore, there is an urgent need to develop ABCB1 inhibitors that are highly efficient and low in toxicity, and which do not have pharmacokinetic interactions with concurrently used chemotherapy drugs.

Danshen, derived from the dried root and rhizome of *Salvia miltiorrhiza Bunge*, a plant in the *Lamiaceae* family, is obtained after harvesting and drying [12]. It is included in the list of items approved by the Ministry of Health of China for dual use in both food and medicine and is suitable for health food products. Danshen is first mentioned in the “Shennong Bencao Jing” as characterized by a bitter taste, slightly cold nature, and attributed to the heart and liver meridians. It is known for its functions of invigorating blood circulation, dispelling stasis, cooling blood, reducing swelling, relieving restlessness, and calming the mind [13]. Danshensu (DSS), a water-soluble component of Danshen, is more stable than other components and can be quickly absorbed into the human body after ingestion [14]. DSS has pharmacological effects including anti-thrombosis, improvement of microcirculation, antibacterial properties, inflammation reduction, inhibition of cell apoptosis, antioxidation, and enhancement of the body's immune response [15, 16], making it widely used in clinical and scientific research.

This study aimed to explore DSS’s capability to counteract MDR in the MCF-7/DO DG-5031X cell line and elucidate its mechanism using fluorescence spectrophotometry and flow cytometry. Further, this study would explore DSS's potential to suppress the ABCB1 efflux pump function in MCF-7/DOX cells, thereby reversing its multidrug resistance. The results of this study would lay a foundational theory for further detailed exploration and potential clinical utilization of DSS.

Materials and methods

Cell culture

MCF-7 cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and seeded in HyClone RPMI 1640 medium (Cytiva, Marlborough, MA, USA) supplemented with 10% fetal bovine serum (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd., Hangzhou, Zhejiang, China) and cultured at 37°C in a humidified atmosphere with 5% CO_2. The cultural medium was changed every two days. MCF-7/DOX cells obtained from Nanjing KeyGen Biotech Co., Ltd., Nanjing, Jiangsu, China) were seeded in the same HyClone RPMI 1640 medium with 10% fetal bovine serum. The induction concentrations of the drug Doxorubicin (DOX) (Wanle Pharma Co., Ltd., Shenzhen, Guangdong, China) were gradually increased from low (0.6 μg/mL) to high (up to 2 μg/mL). Ultimately, DOX was added to the culture system at a final concentration of 1 μg/mL for long-term passage culture to maintain drug resistance. The culture conditions were the same as MCF-7 cells with a drug-free culture period for two weeks before the treatment.

Tetrazolium salt (MTT) assay for drug sensitivity

(1) Determine the inhibitory effects of DSS and DOX on MCF-7 and MCF-7/DOX cells

MCF-7 cells are sensitive to DOX, while MCF-7/DOX cells are not sensitive to DOX. The sensitivities of DOX to MCF-7 and MCF-7/DOX were compared. Briefly, both MCF-7 and MCF-7/DOX cells with good cell morphology and logarithmic growth phase were centrifuged at 600 rpm for 5 mins. After removing the supernatant, the cells were adjusted to 1.1×10^8 cells/L using RPMI 1640 medium containing 10% calf serum. The cells were then inoculated on a 96 well culture plate with 90 μL (1×10^4 cells) per well. At the same time, 10 μL of different concentrations of DOX were added to each well with the final DOX concentrations in MCF-7 cell plate as 0, 0.2, 0.4, 0.8, 1.6, 3.2 μmol/L, and the final DOX concentrations in MCF-7/DOX cell plate as 0, 2.0, 4.0, 8.0, 16.0, 32.0 μmol/L. Four replicates were conducted for each concentration. Wells with a final DOX concentration of 0 μmol/L served as the control group. Additionally, blank wells were set up with only 100 μL RPMI 1640 medium. The plates were incubated in TC2323 CO_2 incubator (Shellab, Cornelius, OR, USA) at 37°C, 5% CO_2, for 48 h. After incubation, 20 μL of 5 mg/mL 3-(4,5-Dimethylthiazol - 2 - yl)- 2, 5- diphenyltetrazolium bromide (MTT) phosphate buffer solution was added to each well to terminate the reaction and incubated for another 4-6 h under the same conditions. 100 μL of DMSO-SDS (Sigma-Aldrich, Inc., St. Louis, MO, USA) solution was added to each well and incubated overnight to dissolve the formed formazan crystals. The plates were then shaken for 5 minutes and the absorbance at 570 nm was measured using a DG-5031 ELISA analyzer (Huadong Electron Tube Factory, Nanjing, Jiangsu, China). The inhibition rate of cell growth at each concentration of DOX was calculated as follows.

$$\text{Inhibition rate} = \frac{\text{1 - average OD of the experimental group}}{\text{average OD of the control group}} \times 100\%$$

The IC_{50} values were calculated using the Bliss method and SPSS 15.0 software (IBM, Armonk, New York, USA) [11]. The resistance multiple was determined based on the IC_{50} values [17].

$$\text{Resistance multiple} = \frac{IC_{50} \text{ of resistant cells}}{IC_{50} \text{ of sensitive cells}}$$

(2) Determination of the effect of DSS on reversing the resistance of MCF-7/DOX cells

The serum-free RPMI 1640 culture medium was diluted to different concentrations. The resistance reversal test was conducted by using DSS (Chengdu Biopurify Phytochemicals Ltd., Chengdu, Sichuan, China) with the dose less than or equal to IC_{10} (the concentration at which the inhibition rate was less than or equal to 10%). Logarithmic growth phase MCF-7 and MCF-7/DOX cells were collected, and the cell concentration was adjusted to 1.25×10^8 cells/L. 80 μL of the cell suspension (containing 1.0×10^4 cells) was seeded into each well of a 96-well plate. Both cell types were divided into six experimental groups, where experimental group

I was added with 10 µL of different concentrations of DSS (0.01, 0.02, 0.04, 0.08 mg/mL) and 10 µL of RPMI-1640 medium and experimental group II was added with 10 µL of different concentrations of DSS and 10 µL of different concentrations of DOX. Four replicates were set for each dose in both experimental groups. Control group I contained no cells, only 100 µL of RPMI-1640 medium for zero adjustment. Control group II was the negative control with only cells and 20 µL of RPMI-1640 medium. Control group III was the anticancer drug control with 10 µL of different concentrations of DOX and 10 µL of RPMI-1640 medium. Control group IV was the positive control for the reversal agent with a final concentration of 10 µmol/L Verapamil (VRP) (10 µL) and 10 µL of different concentrations of DOX. The fold reversal was calculated as follows.

$$\text{The fold reversal (FR)} = \frac{IC_{50} \text{ value before reversal}}{IC_{50} \text{ value after reversal}}$$

Determination of DOX content in MCF-7 and MCF-7/DOX cells using fluorescence spectrophotometry

Log-phase MCF-7 (M) and MCF-7/DOX (M/D) cells were adjusted to a concentration of 6.25 × 10^8 cells/L and seeded in a 24-well plate with 800 µL (5 × 10^5 cells) per well. Each cell type was divided into five groups including M, M + VRP (10 µmol/L), M + DSS (0.02 mg/mL), M + DSS (0.04 mg/mL), M + DSS (0.08 mg/mL), M/D, M/D + VRP (10 µmol/L), M/D + DSS (0.02 mg/mL), M/D + DSS (0.04 mg/mL), M/D + DSS (0.08 mg/mL). Each group had three replicates. Corresponding drug solutions of 100 µL were added to each well, along with 100 µL of DOX to reach a final concentration of 5 µg/mL and a final volume of 1 mL. After mixing, the cells were incubated at 37°C in a 5% CO_2 humidified atmosphere for 1 hour. The cells were then washed three times with cold PBS and resuspended in 1 mL of PBS. Fluorescence intensity of each group was measured using a fluorescence spectrophotometer (Shimadzu, Nakagyō-ku, Kyoto, Japan) with an excitation wavelength of 480 nm and emission wavelength of 570 nm. The relative fluorescence intensity values were calculated. A Coulter EPICS XL flow cytometer (Beckman Coulter, Inc., Brea, California, USA) equipped with an argon ion laser as the excitation source was used. The excitation wavelength was 488 nm, and the maximum emission wavelength was 575 nm. The data were collected on a computer and analyzed for DOX content using Multicycle software [18].

Determination of the effect of DSS on ABCB1 expression in MCF-7/DOX membrane

MCF-7 and MCF-7/DOX cells with good morphology in the log phase were centrifuged at 600 rpm for 5 mins. The cells were resuspended in RPMI 1640 medium containing 10% fetal bovine serum to adjust the cell concentration to 5.6 × 10^5 cells/mL. The cell suspension was inoculated into a 24-well plate with 900 µL (5 × 10^5 cells) per well. The experiment was divided into groups of M, M/D, M/D + 10 µmol/L VRP, M/D + 0.02 mg/mL DSS, M/D + 0.04 mg/mL DSS, M/D + 0.08 mg/mL DSS with four replicates per group. One well served as the control, and three wells were for measurement. Each group received 100 µL of the corresponding drug solution, making a total volume of 1 mL per well. After mixing, the cells were incubated at 37°C in a 5% CO_2, humidified atmosphere for 48 hours. Cells were then collected, and metabolic activity was halted with 2 mL of cold PBS. The cells were centrifuged at 1,500 rpm for 2 mins to remove the supernatant, followed by two washes with 200 µL of cold PBS. The control tube received 15 µL of mouse IgG for zeroing, and the measurement tubes received 15 µL of mouse anti-human ABCB1 monoclonal antibody (Annolen (Beijing) Biotechnology Co., Ltd., Beijing, China). The samples were incubated in the dark for 30 minutes, then 500 µL of PBS was added to each tube and the cells were resuspended. The Coulter EPICS XL flow cytometer equipped with an argon ion laser as the excitation source was employed with the excitation wavelength of 488 nm, and the maximum emission wavelength of 575 nm. Data were collected on a computer and analyzed for

Table 1. DSS and DOX on the inhibition rate of MCF-7 and MCF-7/DOX cells.

Group	DOX (μmol/L)					
	0	2.0	4.0	8.0	16.0	32.0
Negative Control	0	3.5 ± 0.7	5.9 ± 0.6	9.4 ± 0.8	15.5 ± 0.9	25.9 ± 1.1
VRP (10 μmol/L)	0	30.1 ± 1.4	39.9 ± 2.7	49.9 ± 3.4	62.1 ± 3.6	69.9 ± 3.7
DSS (0.01 mg/mL)	0.9 ± 0.3	1.0 ± 0.3	7.6 ± 0.6	11.6 ± 0.7	27.9 ± 1.6	62.2 ± 2.6
DSS (0.02 mg/mL)	2.2 ± 0.5	6.9 ± 0.7	16.8 ± 0.8	27.9 ± 2.2	49.8 ± 3.7	72.8 ± 4.8
DSS (0.04 mg/mL)	2.3 ± 0.6	39.8 ± 3.8	56.7 ± 4.4	65.9 ± 5.1	69.6 ± 5.4	72.6 ± 5.9
DSS (0.08 mg/mL)	3.2 ± 0.5	52.7 ± 3.9	56.8 ± 4.5	63.7 ± 5.6	69.9 ± 6.7	75.1 ± 7.1

Table 2. The inhibitory rate of MCF-7 cell proliferation by the combined action of DSS and DOX.

Group	DOX (μmol/L)					
	0	0.20	0.40	0.80	1.60	3.20
Negative Control	0	1.1 ± 0.3	3.7 ± 0.7	10.9 ± 0.6	40.1 ± 1.5	80.1 ± 5.2
VRP (10 μmol/L)	0	3.2 ± 0.7	9.8 ± 1.1	24.7 ± 1.3	47.2 ± 5.4	82.9 ± 7.8
DSS (0.01 mg/mL)	1.3 ± 0.3	1.0 ± 0.5	3.5 ± 0.6	10.4 ± 1.4	37.6 ± 4.6	80.1 ± 7.7
DSS (0.02 mg/mL)	2.1 ± 0.4	1.0 ± 0.6	3.6 ± 0.7	10.3 ± 2.9	40.2 ± 4.9	80.2 ± 7.9
DSS (0.04 mg/mL)	4.2 ± 0.7	6.2 ± 0.9	6.1 ± 0.9	10.3 ± 1.9	40.3 ± 5.2	73.6 ± 7.8
DSS (0.08 mg/mL)	5.3 ± 0.4	6.3 ± 1.3	9.2 ± 0.8	21.6 ± 2.6	49.9 ± 5.3	77.9 ± 7.6

Table 3. The inhibitory effect of DSS on the proliferation of MCF-7 and MCF-7/DOX cells.

Group	MCF-7		MCF-7/DOX	
	IC_{50} of DOX (μmol/L)	Fold Reversal	IC_{50} of DOX (μmol/L)	Fold Reversal
Control	2.58		79.98	
VRP (10 μmol/L)	2.15	1.2	9.98	8.0
DSS (0.01 mg/mL)	2.43	1.0	35.57	2.3
DSS (0.02 mg/mL)	2.35	1.1	19.98	4.0
DSS (0.04 mg/mL)	2.42	1.0	4.21	21.3
DSS (0.08 mg/mL)	2.32	1.4	2.13	42.1

cell membrane ABCB1 expression levels using Multicycle software [19].

Statistical analysis

SPSS 15.0 software was employed for statistical analysis. All experimental data were presented as mean ± standard deviation and statistically evaluated to determine significant differences *via* the intergroup t-test. For comparisons across multiple groups, One-way ANOVA was employed. *P* value less than 0.05 was considered as indicative of statistical significance.

Results

The inhibitory effects of DSS and DOX on MCF-7 and MCF-7/DOX cells

The inhibition rates of MCF-7/DOX at various concentrations of DOX were shown in Table 1, while the inhibition rates of MCF-7 at different concentrations of DOX were shown in Table 2. The half-maximal inhibitory concentration (IC_{50}) was calculated according to the Bliss method. The results show that the IC_{50} of DOX for MCF-7 was 2.58 μmol/L, while, for MCF-7/DOX, it was

Table 4. The reversal effect of DSS on the drug resistance of MCF-7 and MCF-7/DOX cells.

DSS Concentration (mg/mL)	Inhibition Rate (%)	
	MCF-7	MCF-7/DOX
0.01	1.3 ± 0.3	0.9 ± 0.3
0.02	2.1 ± 0.4	2.2 ± 0.5
0.04	4.2 ± 0.7	2.3 ± 0.6
0.08	5.3 ± 0.4	3.2 ± 0.5

Table 5. The effect of DSS on DOX accumulation in MCF-7 and MCF-7/DOX cells.

Group	Relative Fluorescence Intensity Value	
	MCF-7	MCF-7/DOX
Control	5.25 ± 0.21	1.24 ± 0.25
VRP (10 μmol/L)	5.19 ± 0.23	3.96 ± 0.24
DSS (0.01 mg/mL)	5.21 ± 0.22	1.63 ± 0.30
DSS (0.02 mg/mL)	5.23 ± 0.19	2.15 ± 0.26
DSS (0.04 mg/mL)	5.21 ± 0.27	2.95 ± 0.27
DSS (0.08 mg/mL)	5.22 ± 0.21	3.45 ± 0.21

79.98 μmol/L. The resistance factor of MCF-7/DOX was 31 (Table 3).

The Impact of DSS on the Proliferation of MCF-7 and MCF-7/DOX

The inhibition rates of MCF-7 and MCF-7/DOX cells at DSS concentrations ranging from 0.01 to 0.08 mg/mL were shown in Table 4. The results indicated that the inhibition rates for cell growth within the 0.01 to 0.08 mg/mL range were less than or equal to 5%, suggesting negligible direct cytotoxic effects at these concentrations. Therefore, these concentrations were deemed suitable for use as reversal agent doses.

The reversal effect of DSS on drug resistance in MCF-7/DOX cells

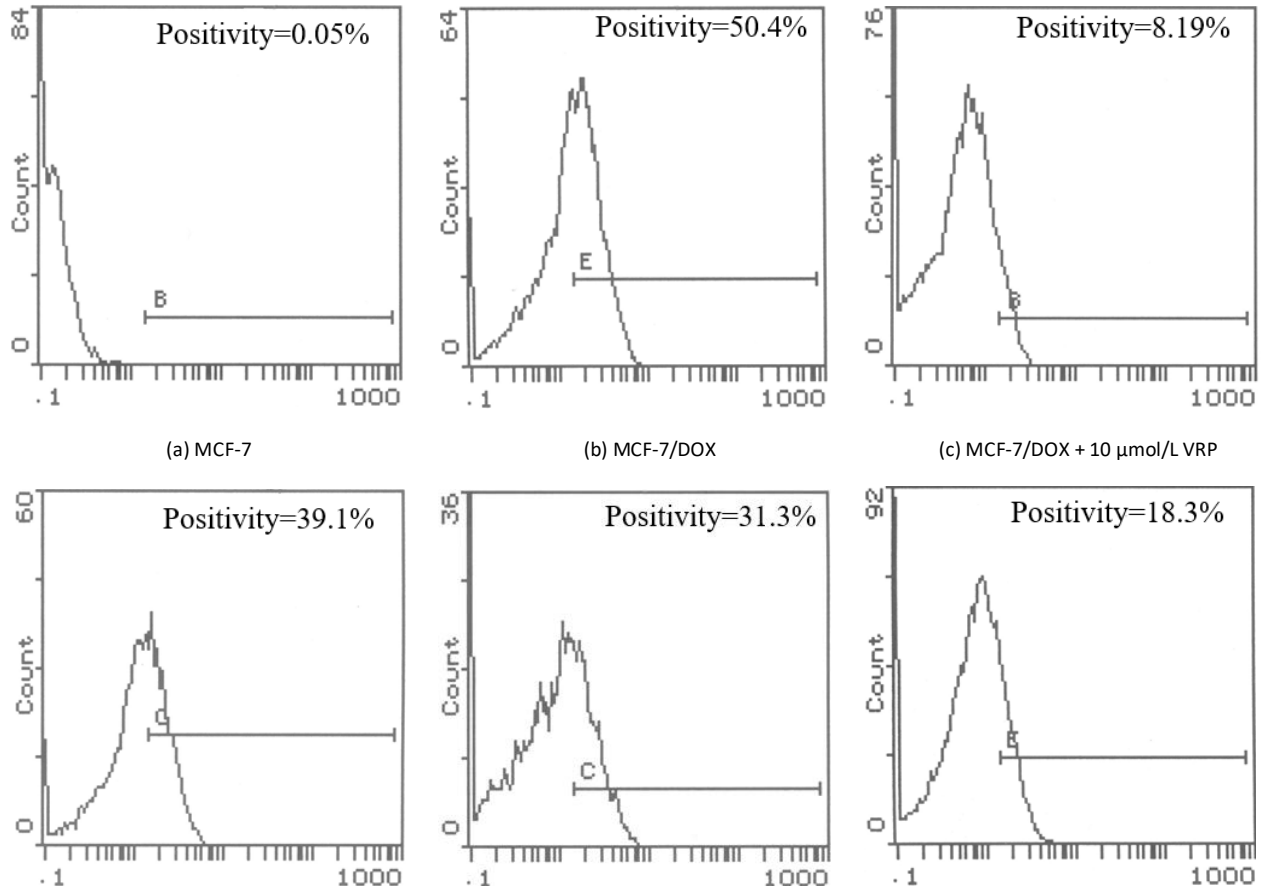
At concentrations of 10 μmol/L VRP and 0.01, 0.02, 0.04, 0.08 mg/mL DSS, the fold reversal of DOX resistance in MCF-7/DOX cells were 8.0, 2.3, 4.0, 21.3, and 42.1, respectively. These results demonstrated that DSS, similar to the reversal agent VRP, could reverse the DOX resistance in MCF-7/DOX cells. Notably, at concentrations of 0.04 and 0.08 mg/mL, the fold reversal by DSS was higher than that by VRP. DSS enhanced the growth inhibitory effect of DOX on MCF-7/DOX cells in a dose-dependent manner, while it had no significant effect on MCF-7 cells.

The effect of DSS on DOX accumulation in MCF-7 and MCF-7/DOX cells

Fluorescence spectrophotometry results showed that, after 1 hour of DOX exposure, the relative intensity value in MCF-7 cells was 5.25 ± 0.21, which was significantly higher than that in MCF-7/DOX (1.24 ± 0.25). After 1 hour of treatment with 10 μmol/L VRP and 0.01, 0.02, 0.04, 0.08 mg/mL DSS, the relative intensity of DOX content in MCF-7/DOX increased from 1.24 ± 0.25 to 3.96 ± 0.24, 1.63 ± 0.30, 2.15 ± 0.26, 2.95 ± 0.27, and 3.45 ± 0.21, respectively (Table 5). The results confirmed that DSS could increase the DOX content in MCF-7/DOX in a dose-dependent manner. However, DSS did not significantly affect the DOX content in MCF-7 cells.

The influence of DSS on ABCB1 expression in the membrane of MCF-7/DOX cells

The flow cytometry results revealed that the ABCB1 positive expression rate in MCF-7 cells was 0.05%, while it was 50.4% in MCF-7/DOX cells. After 48 hours of treatment with 10 μmol/L VRP and 0.02, 0.04, 0.08 mg/mL DSS, the ABCB1



(a) MCF-7 (b) MCF-7/DOX (c) MCF-7/DOX + 10 μmol/L VRP

(d) MCF-7/DOX + 0.02 mg/mL DSS (e) MCF-7/DOX + 0.04 mg/mL DSS (f) MCF-7/DOX + 0.08 mg/mL DSS

Figure 1. Flow cytometric analysis of ABCB1 expression in MCF-7/DOX cells treated with DSS.

positive expression rate in MCF-7/DOX cells decreased from 50.4% to 8.19%, 39.1%, 31.3%, and 18.3%, respectively, corresponding to the reductions of 42.21, 11.3, 19.1, and 32.1 percentage points (Figure 1). Thus, similar to the reversal agent VRP, DSS could reduce ABCB1 expression in both MCF-7/DOX and MCF-7 cells in a dose-dependent effect (Figure 2).

Discussion

Doxorubicin is widely employed in treating human breast cancer and acute leukemia, functioning by inhibiting DNA synthesis within cells. The ability to monitor doxorubicin levels within tumor cells is crucial for predicting treatment effectiveness. However, doxorubicin chemotherapy frequently leads to the development of multidrug resistance (MDR) in tumor cells, often due to enhanced drug efflux that lowers intracellular drug concentrations and results in treatment failure. Consequently, measuring anticancer drug concentrations is a vital component of MDR research [20, 21]. ABCB1, an ATP-dependent drug efflux pump, primarily contributes to cell resistance by altering intracellular drug distribution and transferring drugs out of cells, thus reducing intracellular drug concentrations [22, 23]. It has been confirmed that various drugs, such as VRP and Saracatinib, can inhibit the efflux process of antitumor drugs, increase the accumulation of chemotherapeutic drugs inside tumor cells, and thereby, reverse ABCB1-mediated MDR. Due to its intrinsic fluorescence, DOX serves as an excellent

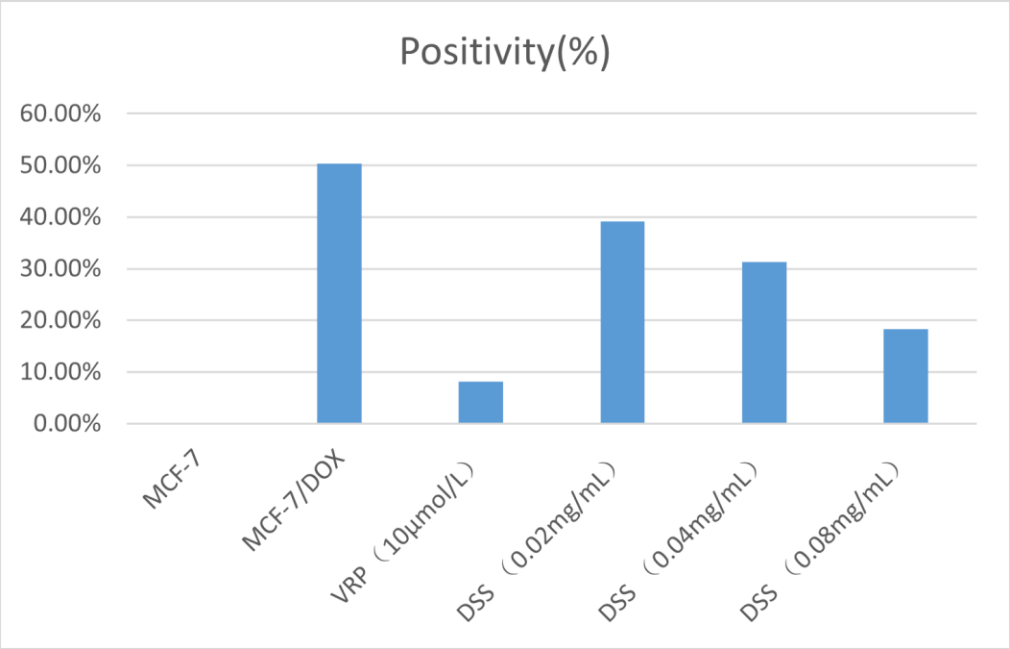


Figure 2. Effect of DSS on the ABCB1 expression in both MCF-7/DOX and MCF-7 cells.

fluorescent indicator of ABCB1 efflux pump function and is widely used to detect the transport action of ABCB1 substrates [24, 25]. The results of this study indicated that, after treatment with DSS, the fluorescence intensity within MCF-7/DOX cells significantly increased, suggesting that DSS could enhance the accumulation of DOX in MCF-7/DOX. Tsuruo *et al*. reported that verapamil (VRP) reversed tumor cell drug resistance and found several chemosensitizers like cyclosporine and trifluoperazine [26]. However, the significant toxic side effects of those chemosensitizers such as cardiotoxicity and nephrotoxicity restricted their clinical use [27]. Some previous studies showed that 6 - 10 μmol/L verapamil was required to fully inhibit ABCB1 during *in vitro* experiments. However, serious cardiovascular toxic side effects may occur at the serum verapamil concentrations of just 1 - 2 μmol/L [28, 29]. While it's recognized that tumor drug resistance arises from complex and multifaceted interactions, the precise mechanisms behind MDR formation in tumor cells and the impact of antitumor drugs on both hematopoietic and non-hematopoietic tissues remain unknown. Currently, there is no ideal clinical strategy for reversing MDR, making the search for new methods to counteract resistance an increasingly critical area of research. Identifying effective reversal agents that minimize toxic side effects is paramount in overcoming MDR. This study confirmed that the combination of non-cytotoxic doses of DSS with the chemotherapeutic drug DOX could reduce the IC_{50} value of MCF-7/DOX cells, showing a positive correlation with the dosage. The non-cytotoxic doses of DSS could also increase the accumulation concentration of doxorubicin in MCF-7/DOX cells, which was also positively correlating with the dosage. Importantly, these non-toxic DSS doses contributed to a significant reduction in ABCB1 protein overexpression in MCF-7/DOX cells in a dose dependent response. It was posited that DSS's ability to modulate ABCB1 overexpression constituted a pivotal mechanism through which it reversed multidrug resistance (MDR).

The potential of DSS to counteract MDR merits further exploration, particularly regarding its effectiveness across a broader spectrum of drug-resistant tumor cells, including human leukemia

K562/DOX cells and human liver cancer BEL-7402/5-FU cells. Future research should also investigate whether DSS can diminish glutathione (GSH) levels in MCF-7/DOX cells and its capability to reverse MDR *in vivo*, thereby elucidating the full therapeutic potential of DSS in overcoming drug resistance.

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